学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

The role of the central amygdala in integrating diverse aversive sensory information
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Various aversive sensory stimuli, such as pain and predator odors, are known to induce negative emotional states, leading to avoidance behaviors and adaptive physiological responses that are essential for survival and maintaining homeostasis. The central amygdala (CeA), which receives inputs from multiple sensory systems, plays a pivotal role in processing stress and coordinating emotional responses. Exposure to aversive pain, olfactory, gustatory, and visual stimuli has been shown to upregulate c-fos mRNA expression and/or c-Fos protein production in the CeA, indicating increased neuronal activity in response to these stimuli. Previous studies have demonstrated that bilateral excitotoxic lesions of the CeA reduce conditioned place aversion induced by noxious stimuli such as intraplantar formalin injection, implicating the CeA in processing the emotional component of pain. Furthermore, optogenetic manipulation has revealed that the neuronal pathway from the parabrachial nucleus (PBN) to the CeA is critically involved in mediating pain-induced aversive states. Despite these advances, it remains unclear whether individual neurons in these brain regions integrate aversive sensory information of diverse modalities (pain, olfactory, and gustatory), or whether distinct neuronal subpopulations convey the aversive sensory information of each modality in parallel. To address this issue, the present study examine the neuronal activation by aversive sensory stimuli of diverse modalities at the single-cell level in the CeA and LPB.

Histological analysis of neuronal activation by two types of aversive stimuli using TRAP2;Ai9 mice

We used TRAP2 mice to investigate whether the individual neurons in the CeA and LPB are activated by two different aversive stimuli. This recombination results in the permanent expression of a reporter gene (e.g.,tdTomato) in the activated neurons, enabling their long-term identification and manipulation. Combination of labeling the neurons activated by the first stimulation using the TRAP2 system and labeling the neurons activated by the second stimulation using immunofluorescence staining enabled to examine whether individual neurons are activated by two different stimuli. For pain stimulation, mice were subcutaneously injected with 10% formalin solution into either hindpaw or whisper pad. For aversive olfactory stimulation, mice were exposed to 2-methyl-2-thiazoline (2MT), a predator odor. For aversive gustatory stimulation, 10 mM quinine solution was infused into the mice’s mouth. The results suggest that TRAP labeling and immunostaining differ in sensitivity, leading to a lack of accurate quantification in the detection of neurons activated by the two types of stimuli. However, the results provide semi-quantitative data on CeA and LPB neurons activated by aversive sensory stimulation of two modalities that the percentages of double-labeled neurons were 8.6%, 10.1%, 20.8%, and 3.8% in the CeA, 13.0%, 12.7%, 12.2%, and 6.2% in the LPB, and 18.8%, 23.1%, 25.0%, and 6.2% in the LPBE, when the first and second stimuli were 2MT and pain

(hindlimb), 2MT and pain (whisker pad), 2MT and bitter taste (quinine), bitter taste (quinine) and pain (whisker pad), respectively.

Ca^{2+} imaging to visualize the activation of CeA neurons by various aversive sensory stimuli

In vivo Ca^{2+} imaging with C57BL/6 J mice injected with AAVDJ-hsyn-GCaMP6m into the CeA to express GCaMP6m in the entire CeA neuros showed that 24.0%, 27.0%, 20.5%, and 5.0% CeA neurons were activated in response to aversive pain, olfactory, and gustatory, and visual stimuli (bright light), respectively. The percentage of neurons activated by one or more sensory stimuli was 54.0%. Since there were considerably fewer neurons that responded to visual stimuli than to the other three types of stimuli, we focused on the 107 neurons that responded to any of the aversive pain, olfactory, or gustatory stimuli. Seventy-five (70.1%), 27 (25.2%), and 5 (4.7%) neurons responded to only one type, two types, and all three types of stimulation, respectively. 29.9% of neurons responded to two or more stimuli among the activated neurons, suggesting that the integration of information in the aversive sensory signaling pathway is only partial up to the CeA neurons.

Ca^{2+} imaging to visualize the activation of PKCδ-positive CeA neurons by various aversive sensory stimuli

In vivo calcium imaging with PKCδ-Cre mice injected with AAVDJ-Ef1a-DIO-GCaMP6m into the CeA to express GCaMP6m selectively in the $PKC\delta^+$ CeA neuros showed that 36.3%, 31.4%, 23.5%, and 5.9% of $PKC\delta^+$ CeA neurons, the subpopulation of neurons located in the CeC/CeL, were activated in response to pain, olfactory, and gustatory (aversive), and gustatory (rewarding) stimuli, respectively. The percentage of neurons activated by one or more sensory stimuli was 70.7%. To compare with the results in C57BL/6 J mice injected with AAVDJ-hsyn-GCaMP6m, among the 72 neurons that responded to any of the aversive pain, olfactory, or gustatory stimuli, the percentage of neurons that responded to only one type of stimulus, to any two types of stimuli, or to all three types of stimuli was determined. Fifty-five (76.5%) neurons responded to only one type of aversive sensory stimulus. Only 23.5% of neurons responded to two or more stimuli, suggesting that the integration of information in the aversive sensory signaling pathway is only partial up to the $PKC\delta^+$ neurons in the CeA. This percentage (23.5%) is lower than the percentage (29.9%) when GCaMP6m was expressed in entire CeA neurons. Considering the position of $PKC\delta^+$ neurons as entry points for sensory information in the CeA, it is possible that the proportion of neurons that respond to two or more stimulus is larger in the entire CeA due to increased integration of aversive sensory information within the CeA.

Conclusion

The present study demonstrated that diverse aversive sensory information is only partially integrated in the CeA or its upstream brain regions such as the LPB. Future studies are needed to examine the response to aversive sensory stimuli in other neuronal populations than $PKC\delta^+$ neurons within the CeA and in the neurons located in the downstream brain regions such as the VTA to clarify the neuronal mechanisms for integration of diverse aversive sensory information to mediate appropriate behavioral and autonomic responses.